Remarks

Reconsideration and withdrawal of the rejections of the claims, in view of the amendments and remarks herein, is respectfully requested. Claims 1, 6-7, 10 and 34 are amended. The amendments are intended to advance the application and are not intended to concede to the correctness of the Examiner's position or to prejudice the claims prior to amendment, which claims are present in a continuation of the above-referenced application. Claims 1-34 are now pending in this application.

The specification is amended at page 8 to address the Examiner's comment at page 2 of the Office Action. Support for the amendment is found at page 8, lines 17-28 of the specification.

The specification is amended at pages 10, 48 and 56 to correct typographical errors. Support for the amendment at page 10 is found at page 56, line 13. Support for the amendment at page 48 is found at page 49, line 3 and support for the amendment at page 56 is found at page 10, lines 5-16.

The 35 U.S.C. § 112, Second Paragraph, Rejection

The Examiner rejected claim 34 under 35 U.S.C. § 112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. The amendment to claim 34 moots the § 112(2) rejection. Thus, withdrawal of the § 112(2) rejection is respectfully requested.

The 35 U.S.C. § 112, First Paragraph, Rejections

The Examiner rejected claims 1-2, 4-18, 27-29, and 34 under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention, and as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor, at the time the application was filed, had possession of the claimed invention. These rejections are respectfully traversed.

In particular, the Examiner asserts that 1) "EuPL" is unclear; 2) Figure 8 does not show cleavage of supercoiled DNA; 3) "singly occupied dimer" is unclear; 4) it is unclear how the phrase "Ca binding motif" relates to Table 1; 5) the values in the last column of Table 1 and their relationship to columns 2-4 are unclear; 6) the numbering of positions in the peptides relative to their parental polypeptides is unclear; and 7) there is no data showing specific binding to a nucleic acid.

Applicant has provided a detailed disclosure of synthetic peptides which contain a domain which binds a metal that is between domains from a region of a protein which binds nucleic acid (page 3, lines 13-17 and page 5, lines 8-10), e.g., see Figure 2 where nucleic acid binding domains are shown with double underlining and metal binding domains are shown with single underlining. In particular, it is disclosed that the helical orientation of a metal binding domain of metal binding proteins may be substantially superimposable on that of a nucleic acid binding domain of nucleic acid binding proteins (page 3, lines 18-25). If the two structures are substantially superimposable, a chimeric peptide may be prepared in which the metal binding domain generally replaces sequences in the nucleic acid binding domain which are, for example, between two helices or between a helix and a strand in the nucleic acid binding region, yielding a chimeric peptide having both metal binding and nucleic acid binding properties (page 4, lines 10-14). For instance, a nucleic acid binding region of Engrailed (a known DNA binding protein, see the key words section in NCBI Accession No. A48423, and Mainguy et al., J. Invest. Dermat., 113:643 (1999), both of record) and a EF-Hand metal binding domain (a known metal binding motif, see abstract of Lewit-Bentley et al., Curr. Opin. Struct. Biol., 10:637 (2000), a copy is enclosed herewith), a nucleic acid binding region of Engrailed and a metal binding domain of calmodulin (loop I) (a known metal binding protein, see the abstract of Zhang et al., Biochem. Cell Biol., 76:313 (1998), a copy is enclosed herewith), and a nucleic acid binding region of Antennapedia (a known DNA binding protein, see the key words section in NCBI Accession No. P02833 and Mainguy et al., both of record) and a metal binding domain of calmodulin (loop III), are substantially superimposable (Figure 2 and page 4, lines 15-20).

With respect to basis 1) of the rejection, "Eu5L" is not mentioned in Applicant's specification although a metal-peptide complex "EuP5L" is referred to in Table 1. The peptide P5L is described at page 51, lines 9-11 of the specification.

To determine whether peptide-metal complexes interact with supercoiled plasmid DNA in a concentration dependent manner, pUC19 plasmid DNA was mixed with various amounts of metal salt or metal-peptide complexes. Lane 1 in Figure 8 is pUC19 plasmid DNA (control), lanes 2-5 of Figure 8 are plasmid DNA with increasing concentrations of $EuCl_3$, lanes 6-9 in Figure 8 are plasmid DNA plus increasing concentrations of free P3, and lanes 10-15 are plasmid DNA plus increasing concentrations of EuP_3 (page 10, lines 10-14). With respect to lanes 2-5, page 2, lines 5-6 of the specification disclose that lanthanides (alone) hydrolyze supercoiled DNA. At page 56, lines 14-16, it is noted that the highest amounts of nicking of DNA by EuP_3 occurred at 30 μM and at page 10, lines 14-16, it is noted that at higher concentrations of EuP_3 (40, 50 and 100 μM; lanes 13-15), significant affinity for DNA decreases total DNA intensity. Thus, Figure 8, as well as Figure 7 and Table 1, show that EuP_3 cleaves DNA. Moreover, Table 1 shows that EuP4a and EuP5L cleave DNA.

In this regard, the Examiner is also requested to consider the Rule 132 Declaration enclosed herewith which is executed by Dr. Sonya Franklin, the inventor of the claims of the present application. In the Declaration, Dr. Franklin explains the data in Figure 8 and provides further evidence that peptides of the invention, in the presence of metal, cleave supercoiled DNA.

With regard to bases 3)-5) of the rejections, at page 1 of the specification, it is noted that homeodomains can bind DNA as a monomer or as a dimer. On page 49, it is disclosed that EuP3 can dimerize but that the second metal site has low affinity and so at concentrations below 100 μM, Eu(III), EuP3 monomer and singly occupied dimer are the species present. EuP3 contains a nucleic acid binding domain (portions of the Engrailed homeodomain) and an EF-Hand metal binding domain, i.e., a Ca^{2+} binding domain (see Brief Description of Figure 2, and page 46, lines 11-17, page 47, line 19, and page 60, lines 22-23). For the first set of data in Table 1 (related to Eu and P3 complexes), columns 2-4 provide the calculated concentration of Eu(free), EuP3 and $EuP3_2$ (the latter is a singly occupied dimer), respectively, based on the concentration of Eu and P3 in column 1 and the dissociation constant for EuP3 (page 48, lines 1-9). Column 5 ("rates") is the rate of cleavage of a model substrate, BNPP, by species present in a particular mix of metal and peptide (page 60, line 1).

The Examiner asserts that Applicant has failed to clarify the numbering of amino acid residues in peptides disclosed the specification in papers filed on January 10, 2003 (which included an Amendment) and February 26, 2004 (which included a Preliminary Amendment filed with a Request for Continued Examination (RCE)).

In this regard, the Examiner is requested to refer to the Rule 116 Amendment filed on December 23, 2003 (the RCE requested consideration of that Amendment) in conjunction with the Preliminary Amendment filed with the RCE. In the Amendment filed on December 23, 2003, Applicant noted that Figure 2 provides the sequence of P3 and indicates whether the sequence is from Engrailed, a nucleic acid binding protein (sequences from Engrailed are indicated by a double underline) or EF-Hand, a calcium binding protein (EF-Hand sequences are indicated by a single underline), i.e., TERRRQQLDKDGDGTI DERE IKIHFQNKRAKIK (see Figure 2). The Engrailed homeodomain is well known to the art (see, for example, NCBI Accession No. B25682, where the homeodomain in *Drosophilia* Engrailed includes residues 487 to 543; NCBI Accession No. A48423, where the homeodomain in murine Engrailed includes residues 313 to 369; and NCBI Accession No. NP-001417, where the homeodomain in human Engrailed includes residues 303 to 362 (a copy of each was enclosed with the Amendment filed on December 23, 2003).

In the Preliminary Amendment filed on January 10, 2003, a copy of NCBI Accession No. NP-001417, which discloses a human Engrailed amino acid sequence, was provided in which the sequences in P3 corresponding to the Engrailed homeodomain were underlined. Note that residue 27 of the homeodomain in human Engrailed (not residue 27 of the full-length protein) is T, the first residue in P3; residue 34 of the homeodomain in human Engrailed is L, which is the eighth residue in P3; residue 42 of the homeodomain in human Engrailed is E, which is the eighteenth residue in P3; and residue 57 of the homeodomain in human Engrailed is K, which is the twenty-second residue in P3. The specification discloses that the first 8 residues of P3 are from the α2 helix of Engrailed (TERRRQQL, i.e., corresponding to residues 27 (residue 27 is a threonine) to 34 (residue 34 is a leucine) (i.e., T_{27}-L_{34}) of the Engrailed homeodomain) and the last 16 residues of P3 are from the α3 helix of Engrailed (ERE...KIK, i.e., corresponding to residues 42 (residue 42 is a glutamic acid) to 57 (residue 57 is a lysine) (i.e., E_{42}-K_{57}) of the Engrailed homeodomain) (page 9, lines 1-2 and page 50, lines 25-28). Moreover, the residue at

the position in P3 corresponding to position 43 in a homeodomain is disclosed as the residue at position 43 in the *Antennapedia* homeodomain, i.e., R (see NCBI Accession No. P02833, where the homeodomain includes residues 297 to 356; a copy was enclosed with the Amendment filed on December 23, 2003).

The following alignment summarizes the numbering of positions in P3, the numbering of the corresponding *Drosophila* Engrailed homeodomain sequences, i.e., the first numbered residue is the first residue in the homeodomain, the numbering of the corresponding *Drosphilia* Engrailed sequences in the full-length protein, the numbering of the corresponding human Engrailed sequences in the full-length protein, and the numbering of the corresponding Antennapedia homeodomain sequences in the full-length protein.

P3	TERRRQQLDKDGDGTIDEREIKIHFQNKRAKIK 1 8 18 33
Homeodomain numbering in B25682	TERRRQQL..(7 residues)...EAQIKIWFQNKRAKIK 27 34 42 57
Numbering of homeodomain in full-length protein in B25682	TERRRQQL..(7 residues)...EAQIKIWFQNKRAKIK 512 519 527 542
Numbering of homeodomain in full-length protein NP001417	TEQRRQTL..(7 residues)...ESQIKIWFQNKRAKIK 329 336 344 359
Numbering of homeodomain in full-length protein P02833	TRRRRIEI ..(7 residues)... ERQIKIWFQNRRMKWK 323 330 338 353

Moreover, as evidenced by i) Pomerantz et al. (Proc. Natl. Acad. Sci. USA, 92:9752 (1995)); Pomerantz et al. (Science, 267:93 (1995); and NCBI Accession No. P8154 (the amino acid sequence of human Oct-1, a transcription factor with a homeodomain at residues 379 to 438 of a 743 amino acid protein); ii) Mainguy et al. (J. Invest. Dermat., 113:643 (1999)); iii) Tejada et al. (DNA Cell Biol., 18:791 (1999)) and NCBI Accession No. Q90655 (the amino acid sequence of AKR, a transcription factor with a homeodomain at residues 35 to 97 of a 269 amino acid protein); and iv) Kim et al. (J. Biol. Inorg. Chem., 6:173 (2001) (a copy of each document not previously submitted is enclosed herewith), residues in a homeodomain are often referenced by their position in the homeodomain rather than in their position in the full-length protein. For instance, at page 9754 of Pomerantz et al. (Proc. Natl. Acad. Sci. USA, 92:9752 (1995)), it is disclosed that mutations were made at positions 11, 18, 22, 30 and 39 of the Oct-1 homeodomain (a transcription factor of greater than 700 residues, and where residues 379-438 human Oct-1

represent a homeodomain). In the abstract of Mainguy et al., it is disclosed that BPAG1 is a protein which is a putative transcriptional target of homeoproteins with a glutamate (Q) at position 50 of their homeodomain (see alignment above in which all three full-length proteins have a Q at position 50 of the homeodomain), while in the abstract of Tejada et al. AKR is disclosed as a protein with a highly divergent Ile residue at position 50 of the DNA-recognition helix (homeodomain). Thus, the numbering of peptide residues in Applicant's specification adequately describes and enables the claimed invention.

Moreover, the Examiner has failed to consider Kovacic et al. (J. Am. Chem. Soc., 125:6656 (2003)), Caravan et al. (Chem. Comm., 21:2574 (2003)), and Sirish et al. (J. Inorg. Biochem., 91:253 (2002)) (a copy of each was enclosed with the Amendment filed December 23, 2003) which disclose metal complexes with peptides of the invention other than EuP3, EuP4a or EuP5L, e.g., CeP3W, EuP3W, CeP4, EuP4 and GdP3W. In particular, Sirish et al. disclose that P4 and P3W bound metal and both peptides were found to cleave DNA. The Examiner is respectfully reminded that Applicant is entitled to submit post-filing date evidence of enablement. In re Wands, 8 U.S.P.Q.2d 1400, 1406 (Fed. Cir. 1988); In re Ambruster, 185 U.S.P.Q. 152 (C.C.P.A. 1975).

With regard to the term "specifically" as it modifies "binds a nucleic acid sequence", the Examiner is requested to consider that the claims are directed to a chimeric peptide having at least two sequences (domains) that specifically bind nucleic acid such as sequences from proteins known to bind DNA, e.g., DNA binding domains of a transcription factor (see, e.g., page 4, lines 5-14 and page 5, lines 3-13 of the specification). The Examiner is also requested to reconsider that the specification discloses that DNA gel shift assays showed that the metallated peptide of the invention bound a plasmid with at least 11 partial target sequences for Engrailed, while a control peptide did not (page 54, lines 21-24 and page 55, lines 9-10). The Examiner is also requested to consider that Kovacic et al., *supra*, relate that CeP3W cleaves DNA with some sequence specificity.

It is respectfully submitted that the pending claims and specification are in conformance with § 112(1). Hence, withdrawal of the § 112(1) rejections is appropriate and is respectfully requested.

Conclusion

Applicant respectfully submits that the claims are in condition for allowance, and notification to that effect is earnestly requested. The Examiner is invited to telephone Applicant's attorney at (612) 373-6959 to facilitate prosecution of this application.

If necessary, please charge any additional fees or credit overpayment to Deposit Account No. 19-0743.

Respectfully submitted,

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Date July 2, 2004

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Reg. No. 39,665

CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail, in an envelope addressed to: Commissioner of Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on this 2nd day of July, 2004.

Dawn M. Poole
Name

Dawn M. Poole
Signature